

## PROBIOTICS IN FOOD AND HEALTH - CURRENT PERSPECTIVE

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### Abstract

Advances in food science and technology are providing the food industry with increasingly effective techniques to control and improve the physical structure and the chemical compositions of the food products, creating functional foods incorporating 'Probiotics' that provide potential attributes beyond basic nourishing properties. At present, the probiotics are at nascent stage specifically in developing countries, but the awareness and knowledge about their natural sources, potential applications and technology to prepare them in a suitable form have to be imparted in the Indian locality as an affordable product for domestic utilization. The review summarizes magnitude of Indian traditional fermented food as a magnificent source of probiotic lactic acid bacteria, current status and future prospective of probiotics in food and health, as well as regulatory framework related to probiotics across the globe.

**Keywords:** Probiotics; Lactic acid bacteria; Health; Food safety; legislation; regulation.

Received: 25.07.2019

Reviewed: 11.10.2019

Accepted: 11.11.2019

## 1. INTRODUCTION

In short span, probiotics have expanded into an integral part of the complex world as biologics, pharmaceuticals as well as food and nutritional supplements owing to their potential of tendering health advantages. These acts as food elements as well as defensive or curative drugs which hold live non-pathogenic bacteria. It is primarily the bacteria and their metabolites produced which impart these probiotics their health promoting properties. Currently, there has been a growing alertness among Indian consumers about the magnitude of nutrition, health and quality of food they eat. Regular exploitation of probiotics could perk up the quality of life and reduces reliance on drugs and medical expenses. Thus efforts are raised up to appraise the health benefits of probiotics in food and pharmaceutical sectors by utilizing existing knowledge to boom Indian market for probiotics (Prajapati, 2015).

## 2. PRESENT STATUS OF PROBIOTICS IN INDIA

India has a long tradition of using foods for

health promoting or functional properties, influenced by *Ayurvedic* medicine. These functional foods consist of dairy and non-dairy products, herbal extracts, spices, fruits and nutritionally improved foods. With its strong tradition of healthful eating, India ranks amongst the top ten nations in buying functional foods (Prajapati, 2015). In the Indian sub-continent, the concept of using probiotics and synbiotics was probably known before 7000 years, as we see many depiction of Lord Krishna in Hindu mythology promoting dahi and buttermilk as well as well-designed synbiotic recipe '*Panchamrut*' in every Hindu ritual (Prajapati and Nair, 2008). However, the scientific awareness of using probiotics and prebiotics in foods increased in last few decades, mainly after the publication of book '*Prolongation of life*' by Metchnikoff in 1908. The probiotic industry in India is an estimated INR 20.6 million with a projected annual growth rate of 22.6% until 2015 (ICMR-DBT, 2011). India's probiotic market is highlighted as a 'major growth market of the future' because of increasing youth population, a growing interest in health care and need for preventive medicine. The major players in the

probiotic category in India are four FMCG (Fast Moving Consumer Goods) giants viz., Amul, Nestle, Mother Dairy and Yakult Danone India Pvt. Ltd. Major pharmaceutical companies have become active and are trying to formulate newer products, drugs and packaged products like probiotic-based nutritional supplements with special needs such as pregnancy, lactation, immunodeficiency etc. and products especially for pediatric and geriatric patients. Some probiotic based pharmaceutical formulations are present viz. Sporolac (Sporolactobacilli), Darolac, Biglac, Bifilac, etc regarding this aspect. The latest addition to the list of probiotics in India is ViBact (which is made up of genetically modified *Bacillus mesentericus*), which acts as an alternate to B-complex capsules (Suvarna and Bobby, 2005). Moreover, dried probiotic formulations and various dosage forms like tablets, capsules and sachets with higher shelf-life, which can be used as food ingredient or inocula for preparation of probiotic fermented milk are also reported (Sreeja and Prajapati, 2015).

Probiotics have various mechanisms of action although the exact manner in which they exert their effects is still not fully elucidated. Figure 1 summarizes the detail mechanisms of action of probiotics to treat various diseases/disorders.

### **2.1. Traditional Indian fermented foods as a natural source for isolation of Probiotics**

India being a large country displays climatic, ethnic and religious diversities vs. variation in food production and consumption. Lactic acid bacteria (LAB) have contributed in increased volume of fermented foods globally especially in foods containing probiotics or health promoting bacteria (Steinkraus, 1996). A lot of diversity prevails in the food habits of the people living in different regions of the country. Isolation and screening of microorganisms from naturally occurring processes have always been the most powerful means for acquiring valuable cultures for scientific and commercial purposes (Patel, 2012; Thakkar et al, 2015). These traditional fermented foods hold true LAB, which are used

throughout the world for the manufacturing of various probiotic functional foods; new isolates may pave a way for intense research in the medical application. Furthermore, large proportions of fermented foods are unexplored for their microbiota, hence these can be exploited in future to isolate functional probiotic strains. Table 1 shows variety of fermented food products of different origin that serve as carriers for probiotic bacteria.

Overall, the major points to be addressed while including probiotics into foods are the selection of a compatible probiotic strain/food type combination as well as food processing conditions that are compatible with probiotic survival (Sanders and Marco, 2010); ensuring that the food matrix supports probiotic growth; selecting a product matrix, packaging and environmental conditions to ensure ample probiotic survival over the product's supply chain and during storage; and at last ensuring that adding up of the probiotic does not adversely impact on the texture and taste of the product (Ranadheera et al., 2010). Technological and functional properties, besides sensory characteristics are the foremost criteria for the success of these products in the market (Rouhi et al., 2013). The functional attributes of dairy and non-dairy probiotic products are further boosted by adding prebiotics such as galacto-oligosaccharide, fructo-oligosaccharide and inulin.

A number of dairy food products including frozen fermented dairy desserts, spray-dried milk powder, cheeses, ice cream, yoghurt, freeze-dried yoghurt have been recommended as delivery vehicles for probiotic to consumer. However, high prevalence of lactose intolerance, different non-dairy probiotic products such as cereal-based products, fruit juices, vegetable juices, soya-based products, oat-based desserts, confectionary products, breakfast cereals and baby foods have been developed in recent years (Nagpal et al., 2012b; Matias et al., 2014). Development of novel, economical and technological matrices is a dire need to bring the non-dairy probiotic foods on par with the demand as their nature of healthy alternatives to dairy probiotic foods (Patel, 2017; Martins et al., 2013).

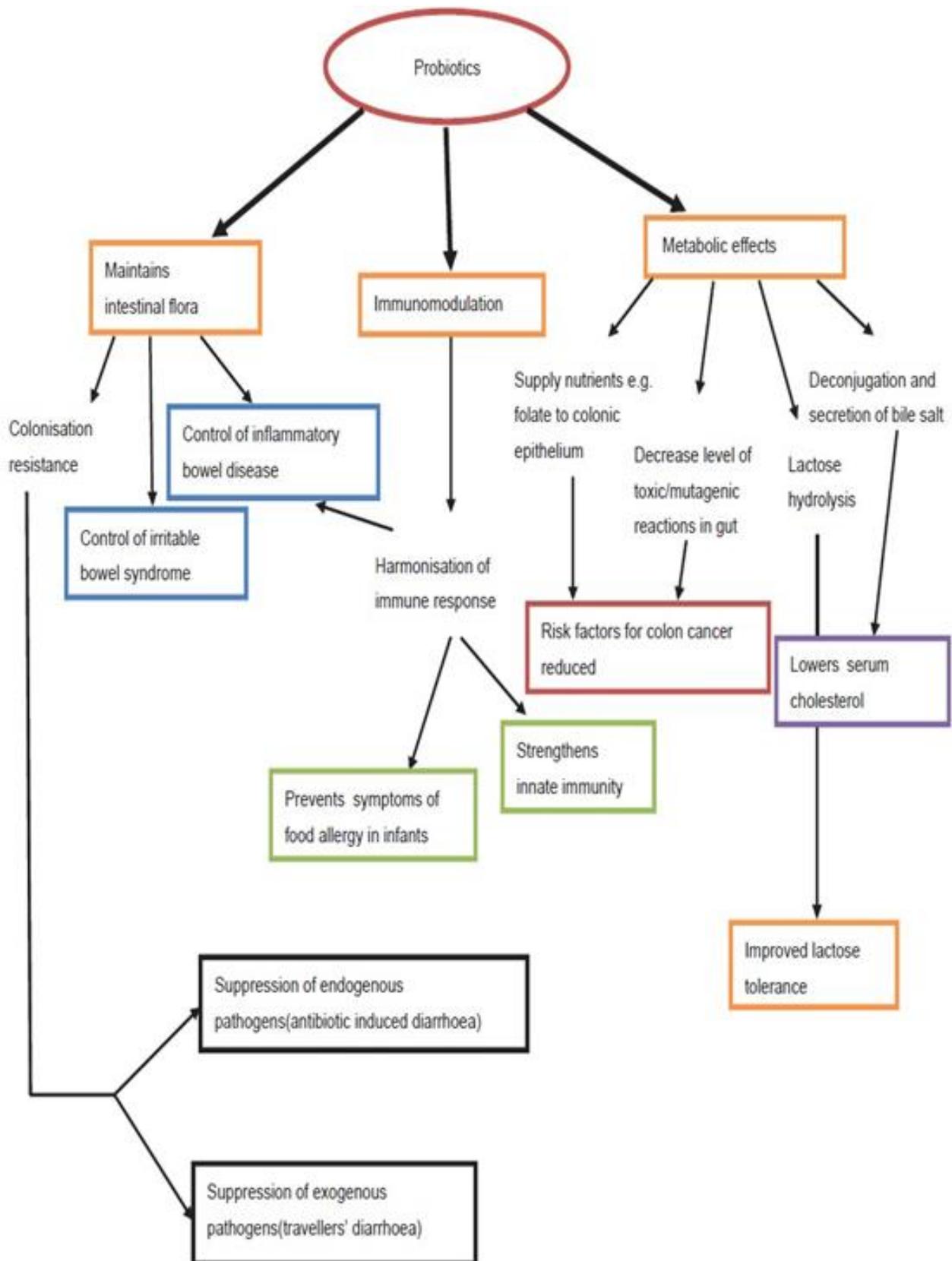


Figure 2 Guidelines for evaluation of candidate probiotic strains (Source: Ganguly et al., 2011).

\*Only required if a specific health claim is made

**Table 1: Commonly utilized fermented food products that serve as carriers for probiotics**

Carrier	Products	Probiotics	References
Dairy Based	Sweet-acidophilus milk	<i>L. gasseri</i>	Usman and Hosono (1999 a)
	Ice-cream	<i>L. johnsonii</i>	Alamprese et al., (2002)
	Carbonated probiotic fermented milk	<i>L. helveticus</i> MTCC 5463 and dairy starter culture <i>S. thermophilus</i> MTCC 5460	Shah and Prajapati (2013)
	Pearl millet based functional fermented skim milk	<i>L. rhamnosus</i> RS13 and <i>S. thermophilus</i> ST20 as starter culture	Basu et al., (2011)
	Herbal probiotic lassi with 1% safed musli powder	<i>L. acidophilus</i> V3 and <i>S. thermophilus</i> MD2	Momin and Prajapati (2009)
	Probiotic beverage with germinated pearl millet flour (GPMF) and liquid barley malt extract (LBME)	<i>L. acidophilus</i> NCDC 13	Ganguli and Sabikhi (2012)
	Whey drink	<i>L. casei</i>	Drgalic et al., (2005)
	Whey cheese	<i>B. animalis</i> , <i>L. acidophilus</i> , <i>L. brevis</i> , <i>L. paracasei</i>	Madudeira et al., (2005)
	Edam cheese	<i>Bifidobacterium bifidum</i> (ATCC 15696)	Sabikhi and Mathur (2002)
	Natural set-yogurt	<i>L. acidophilus</i> , <i>L. casei</i> , <i>Bifidobacterium</i> sp.	Donkor et al., (2007)
	Low fat cheddar cheese	<i>L. casei</i>	Sharp et al., (2008)
	Yogurt	<i>L. acidophilus</i> , <i>L. casei</i> , <i>B. bifidum</i>	Sendra et al., (2008)
	Cheese	<i>Lc. lactis</i> subsp. <i>cremoris</i> , <i>Lc. lactis</i> subsp. <i>lactis</i> , <i>L. delbrueckii</i> subsp. <i>delbrueckii</i> , <i>L. delbrueckii</i> subsp. <i>lactis</i> , <i>L. helveticus</i> , <i>L. casei</i> , <i>L. plantarum</i> , <i>L. salivarius</i> , <i>Leuconostoc</i> , <i>Strep. thermophilus</i> , <i>Ent. durans</i> , <i>Ent. faecium</i> , and <i>Staphylococcus</i> spp., <i>Brevibacterium linens</i> , <i>Propionibacterium freudenreichii</i>	Quigley et al., (2011)
	Srikhand	<i>Lc. lactis</i> subsp. <i>lactis</i> , <i>Lc. lactis</i> subsp. <i>diacetylactis</i> , <i>Lc. lactis</i> subsp. <i>cremoris</i> , <i>Strep. thermophilus</i> , <i>L. delbruecki</i> subsp. <i>bulgaricus</i>	Sarkar (2008); Singh and Singh (2014)
Dahi/ Curd	<i>L. paracasei</i> , <i>Lc. lactis</i> , <i>Strep. cremoris</i> , <i>Strep. lactis</i> , <i>Strep. thermophilus</i> , <i>L. bulgaricus</i> , <i>L. acidophilus</i> , <i>L. helveticus</i> , <i>P. acidilactici</i> , <i>W. cibaria</i> , <i>L. fermentum</i> , <i>L. delbrueckii</i> subsp. <i>indicus</i> , <i>Saccharomyces</i> sp., <i>Candida</i> sp.	Thakkar (2016); Patel et al., (2012a); Patil et al., (2010); Arvind et al., (2010), Harun-ur-Rashid et al., (2007); Yadav et al., (2007 a); Agarwal and Bhasin (2002)	
Khadi	<i>Pediococcus</i> sp.	Sukumar and Ghosh (2010)	
Soy based	Soy milk	<i>Lactobacillus</i> , <i>Bifidobacterium</i> , <i>Streptococcus thermophilus</i>	Donkor et al., (2007)
	Soy cream cheese	<i>L. acidophilus</i>	Liong et al., (2009)
	Soy milk	<i>L. acidophilus</i> , <i>L. casei</i> , <i>Bifidobacterium</i>	Yeo and Liong (2010a)
	Soy milk, soycurd	<i>L. rhamnosus</i> <i>L. acidophilus</i> , <i>L. gasseri</i>	Thakkar (2016); Roopashri and Vardaraj (2009)

	Soy milk, Soy yogurt	<i>L. plantarum</i>	Bedani et al., (2014); Bao et al., (2011)
	Kinema	<i>B. subtilis</i> , <i>B. licheniformis</i> , <i>B. cereus</i> , <i>B. circulans</i> , <i>Ent. faecium</i> , <i>Cand. parapsilosis</i> , <i>Geotrichum candidum</i>	Sarkar et al., (2002); Tamang (2003); Singh et al., (2007)
	Miso	<i>P. acidilactici</i> , <i>Leuc. paramesenteroides</i> , <i>P. halophilus</i> , <i>Streptococcus</i> sp., <i>Asp. Oryzae</i>	Sugawara (2010); Asahara et al., (2006)
	Natto	<i>B. subtilis</i> ( <i>natto</i> )	Nagai and Tamang (2010)
	Wadis	<i>E. faecium</i> , <i>L. fermentum</i> , <i>L. bulgaricus</i> , <i>Streptococcus thermophilus</i> , <i>P. pentosaceus</i> <i>P. acidilactici</i> .	Aidoo et al., (2006); Sandhu and Soni (1989)
Juice based	Tomato juices	<i>L. casei</i> A4, <i>L. delbrueckii</i> D7, <i>L. acidophilus</i> LA39, <i>L. plantarum</i> C3	Yoon et al., (2004)
	Cabbage juices	<i>L. plantarum</i> C3, <i>L. casei</i> A4, <i>L. delbrueckii</i> D7	Yoon et al., (2006)
	Beet juice	<i>L. plantarum</i> , <i>L. casei</i> , <i>L. acidophilus</i>	Yoon et al., (2006)
	Orange and Pineapple juice	<i>L. casei</i> , <i>L. rhamnosus</i> GG, <i>L. paracasei</i> , <i>L. acidophilus</i> LA39	Sheehan et al., (2007)
	Carrot juice	<i>B. lactis</i> Bb12, <i>B. bifidum</i> B7.1	Kun et al., (2008)
	Orange and grape juice	<i>L. plantarum</i> , <i>L. acidophilus</i> , <i>L. fermentum</i>	Nagpal et al., (2012 b)
Cereal and pulse based	Boza (Wheat, rice, maize-Fermented cereals)	<i>L. plantarum</i> , <i>L. acidophilus</i> , <i>L. fermentum</i> , <i>Leuconostoc reffinolactis</i> , <i>Leuconostoc mesenteroides</i> , <i>L. brevis</i> , <i>Saccharomyces cerevisiae</i> , <i>Candida tropicalis</i> , <i>Geotrichum penicillatum</i>	Blandino et al., (2003)
	Mahevu (Fermented maize beverage)	<i>Lactococcus lactis</i> subsp. <i>lactis</i>	Blandino et al., (2003); McMaste et al., (2005)
	Dosa	<i>Leuc. mesenteroides</i> , <i>Ent. faecalis</i> , <i>Tor. candida</i> , <i>Trichosporon pullulans</i>	Thakkar (2016); Patel et al., (2012a); Pal et al., (2005); Aidoo et al., (2006); Battacharya and Bhat (1997)
	Dhokla	<i>Leuc. mesenteroides</i> , <i>L. fermentum</i> , <i>Ent. faecalis</i> , <i>Tor. candida</i> , <i>Tor. Pullulans</i>	Patel et al., (2012a); Moktan et al., (2011); Blandino et al., (2003); Aliya and Geervani (1981);
	Idli	<i>Leuc. mesenteroides</i> , <i>L. delbrueckii</i> , <i>L. fermenti</i> , <i>L. coryniformis</i> , <i>P. acidilactis</i> , <i>P. cerevisiae</i> , <i>Streptococcus</i> sp., <i>Ent. faecalis</i> , <i>Lc. lactis</i> , <i>B. amyloliquefaciens</i> , <i>Cand. cacaoi</i> , <i>Cand. fragicola</i> , <i>Cand. glabrata</i> , <i>Cand. kefir</i> , <i>Cand. pseudotropicalis</i> , <i>Cand. sake</i> , <i>Sacch. cerevisiae</i> ,	Moktan et al., (2011); Sridevi et al., (2010); Sarkar et al., (2002); Radhakrishnamurthy et al., (1961); Steinkraus (1996)
	Bhaturu or indigenous bread	<i>L. plantarum</i> , <i>L. acidophilus</i> , <i>L. lactis</i> , <i>L. mesenteroides</i>	Kanwar et al., (2007);Thakur et al., (2004);
	Jilebi	<i>L. fermentum</i> , <i>L. buchneri</i> , <i>Lc. lactis</i> , <i>Ent. faecalis</i> , <i>Sacch. cerevisiae</i> , <i>Streptococcus lactis</i>	Thakkar (2016); Macfarlane et al., (2006); Prakash et al., (2004) Steinkraus, (1996)
	Raabadi	<i>P. acidilactici</i> , <i>Bacillus</i> sp., <i>Micrococcus</i> sp., Yeasts	Basu et al., (2011); Blandino et al., (2003)
	Ambeli (fermented ragi)	<i>Leuc. mesenteroides</i> , <i>L. fermentum</i> , <i>S. faecalis</i>	Ramakrishnan (1993)
	Sourdough	<i>L. casei</i> , <i>L. delbrueckii</i> , <i>L. plantarum</i> , <i>L. reuteri</i> , <i>L. johnsonii</i>	de Vuyst et al., (2009)

Vegetable based	Kimchi	<i>Leuc. mesenteroides</i> , <i>Leuc. kimchii</i> , <i>W. kimchii</i> , <i>W. cibaria</i> , <i>L. plantarum</i> , <i>L. sakei</i> , <i>L. delbrueckii</i> , <i>L. buchneri</i> , <i>L. brevis</i> , <i>L. fermentum</i> , <i>P. acidilactici</i> , <i>Lc. Lactis</i> , yeasts species of <i>Candida</i> , <i>Halococcus</i>	Chang et al., (2008); Nam et al., (2009); Jung et al., (2011)
	Olives	<i>Leuc. mesenteroides</i> , <i>P. pentosaceus</i> , <i>L. pentosus</i> , <i>L. plantarum</i> , <i>Pseudomonas</i> sp., <i>Sphingomonas</i> sp./ <i>Sphingobium</i> sp./ <i>Sphingopyxis</i> sp.) and yeasts ( <i>Candida</i> cf. <i>apicola</i> , <i>Pichia</i> sp., <i>Sacch. cerevisiae</i> )	Abriouel et al., (2011)
	Sauerkraut	<i>Leuc. mesenteroides</i> , <i>P. Pentosaceus</i> , <i>L. brevis</i> , <i>Lb. plantarum</i> , <i>L. sakei</i>	Kingston et al., (2010); Gupta and Prakash, (2009)
	Sinki (Raddish tap root)	<i>L. plantarum</i> , <i>L. brevis</i> , <i>L. casei</i>	Tamang et al., (2005)
	Khalpi (cucumber)	<i>L. brevis</i> , <i>L. plantarum</i>	Tamang and Tamang (2010)
	Tuaithur (Bamboo shoot)	<i>L. plantarum</i> , <i>L. brevis</i> , <i>P. pentosaceus</i> , <i>Lc. lactis</i> , <i>Bacillus circulans</i> , <i>B. firmus</i> , <i>B. sphaericus</i> , <i>B. subtilis</i>	Chakraborty et al., (2014)
Other non-dairy	'Sorghurt'	Not identified	Sanni et al., (2013)
	Pseudo cereals (amaranth, buckwheat)	<i>L. plantarum</i> , <i>L. acidophilus</i>	Monika et al., (2013)
	As an edible film on pan bread	<i>L. rhamnosus</i> GG	Soukoulis et al., (2014)
	Dry-fermented sausages	Not identified	Sidira et al., (2014)
	Oat based synbiotic drink	<i>L. plantarum</i> B28	Angelov et al., (2006)
	Meat based products	<i>L. reuteri</i> , <i>B. longum</i>	Ammor and Mayo (2007)

Moreover, cereals may act as source of prebiotics- non-digestible carbohydrates, promoting the growth of *Lactobacilli* and *Bifidobacteria* present in the colon. Another good raw material to be used as an alternative for non-dairy probiotic carrier is soy. Lactic acid fermentation, which can be combined with supplemental sucrose, glucose and lactose, is the best way to improve the sensory quality of soymilk and also to mask effects of undesirable compounds (Bedani et al., 2013). Table 2 shows commercial probiotic strains and their manufacturers.

### 3. MICROBIOLOGICAL CONSIDERATIONS FOR PROBIOTIC SELECTION

An important aspect limiting the availability of new probiotic cultures is linked to the industrial costs of detection, characterization, and clinical validation of new candidate LAB strains of probiotic interest (Patel, 2012). This

led to the expansion of different sets of simple *in vitro* screening tests. Table 3 shows the key and desirable criteria for the selection of probiotics in commercial applications.

Many *in vitro* tests are performed when screening for potential probiotic strains. The initial step in the selection of a probiotic LAB strain is the determination of its taxonomic classification, which may give an indication of the origin, habitat and physiology of the strain. LAB is associated with habitats that are rich in nutrients, for example various food products and plant materials. They can be found in soil, water, manure, sewage, and silage and can ferment or spoil food. Particular LAB is inhabitants of the human oral cavity, the intestinal tract, and the vagina, and may have beneficial influence on these human ecosystems. All these characteristics have significant consequences on the selection of the novel strains (Morelli, 2007).

**Table 2: Commercially available (characterized) probiotic strains and their manufacturers** (adapted and modified from Tiwari et al., 2012; Vasudha and Mishra, 2013)

Strain	Commercial products	Source
<i>L. rhamnosus</i> GG, <i>L. rhamnosus</i> 271, <i>L. casei</i> and with <i>L. acidophilus</i> alone or together with <i>Bifidobacterium spp.</i>	Probiotic buttermilk, Prolife dahi, probiotic lassi and Flavyo fruit yogurt	Amul (India)
<i>L. helveticus</i> MTCC 5463 <i>L. rhamnosus</i> MTCC 5462	Sold as ingredient, Probiotic lassi	Anand Agricultural University (AAU) (India)
<i>L. casei</i> strain Shirota	Yakult	Yakult Danone (India)
<i>L. acidophilus</i> NCFM <i>B. lactis</i> HN019 (DR10) <i>L. rhamnosus</i> HN001 (DR20)	Sold as ingredient	Danisco (Madison WI)
<i>L. casei</i> Shirota <i>B. breve</i> strain Yakult	Yakult	Yakult (Tokyo, Japan)
<i>L. casei</i> DN-114 001 (" <i>L. casei</i> Immunitas")	DanActive fermented milk	Danone (Paris, France)
<i>B. animalis</i> DN173 010 (" <i>Bifidis regularis</i> ")	Activia yogurt	Danone (Tarrytown, NY)
<i>B. lactis</i> Bb-12	Good Start Natural Cultures infant formula	Nestle (Glendale, CA)
<i>L. acidophilus</i> LA5	Sold as ingredient	Chr. Hansen (Milwaukee WI)
<i>L. johnsonii</i> Lj-1 (same as NCC533 and formerly <i>L. acidophilus</i> La-1)	LC1	Nestlé (Lausanne, Switzerland)
<i>L. acidophilus</i> La-1	Nestavia Dahi (Low fat product), Cultured milk	Nestle (India)
<i>L. plantarum</i> 299V	Sold as ingredient; Good Belly juice product	Probi AB (Lund, Sweden); NextFoods (Boulder, Colorado)
<i>L. rhamnosus</i> 271	Sold as ingredient	Probi AB (Lund, Sweden)
<i>L. rhamnosus</i> GG ('LGG')	Culturelle; Dannon Danimals	Valio Dairy (Helsinki, Finland) The Dannon Company (Tarrytown, NY)
<i>Lactobacillus paracasei</i> 33	Sold as ingredient	GenMont Biotech (Taiwan)
<i>L. fermentum</i> VRI003 (PCC)	Sold as ingredient	Probiomics (Eveleigh, Australia)
<i>L. rhamnosus</i> R0011 <i>L. acidophilus</i> R0052	Sold as ingredient	Institute Rosell (Montreal, Canada)
<i>L. reuteri</i> MM53	Rela (Fruit Juice)	Biogaia (Sweden)
<i>L. rhamnosus</i> GG	Gefilus fruit drinks	Valio Ltd. (Norway)

The initial screening and selection of probiotics includes testing of the following important criteria: phenotype and genotype stability, including plasmid stability; carbohydrate and protein utilization patterns; acid and bile tolerance and survival and growth; intestinal epithelial adhesion properties; production of antimicrobial substances; ability to inhibit known pathogens, spoilage organisms, or both; immunogenicity; and antibiotic resistance patterns. The ability to adhere to the intestinal mucosa is one of the most vital selection criteria for probiotics because adhesion to the intestinal mucosa is considered to be a prerequisite for colonization. So, the host must

be immuno-tolerant to the probiotic. On the other hand, the probiotic strain can act as an adjuvant and stimulate the immune system against pathogenic microorganisms. It goes without saying that a probiotic has to be harmless to the host: there must be no local or general pathogenic, allergic or mutagenic/carcinogenic reactions provoked by the microorganism itself, its fermentation products or its cell components after decrease of the bacteria (Desai, 2008). Table 3 summarizes all important desirable criteria for the selection of potent probiotics for commercial applications.

**Table 3 Key desirable criteria for the selection of probiotics for Industrial applications** (adapted and modified from modified from Klaenhammer and Kullen 1999; Vasiljevic and Shah, 2008)

Properties of Probiotic Strain	Remarks
Human origin for prospective human use	Even though probiotic yeast <i>Saccharomyces boulardii</i> is not of human origin, this criterion is important for species dependent health beneficiary activities
Acid and bile tolerance; resistance to digestive enzymes-pepsin, pancreatin, etc.; antimicrobial activity against potentially pathogenic bacteria	Essential criteria for oral consumption of probiotics although it may not be for other applications for survival through the intestine, maintaining adhesiveness and metabolic activity; to inhibit foodborne pathogens within the gut
Adhesion to mucosal surface-gastrointestinal tract	Vital to improve immune system, maintain metabolic activity, compete with pathogens by avoiding their adhesion and colonization on mucosal surfaces
Safe for food and clinical application	Precise taxonomic identification and characterization of strains including tests for virulence factors- toxic effects, metabolic activity and inherent properties like infectivity, pathogenicity and antibiotic resistance
Clinically validated and documented health effects	For each particular strain, the minimum effective dosage has to be known according to different products. Placebo controlled, double-blinded and randomized studies should be conducted
Good technological properties	Desired viability or survival during product processing and storage if viable organisms are obligatory, strain stability, phage resistance, oxygen resistance, culturable at large scales, has no negative influences on product flavor or body-texture

### 3.1. Microbiological considerations for health claims

Probiotic can be commercialized either as nutritional supplement, pharmaceutical preparation or food products. To establish as a pharmaceutical product, requires significant time, complex and costly research (involving clinical trials), and expression of well-defined therapeutic targets (Figure 2). The selection of appropriate strains, poorly regulated probiotic quality, human biological factors which impair probiotic viability and difficulties in maintaining new bacterial population in the gut are the few major obstacles in providing probiotic therapy (Tamboli et al., 2003).

### 3.2. Microbiological considerations for safety aspects

In recognition of the importance of assuring safety, even among a group of bacteria that possess GRAS status, assessment of safety of a probiotic should be based upon the following documents.

1. Conducting toxicity or pathogenicity measurements in validated laboratory or animal models that are relevant to the species being considered, as needed (CAST, 2007).

2. Determination of antibiotic resistance patterns (Pael, 2012).

3. Evaluation of certain metabolic activities viz. D-lactate production and bile salt deconjugation.

4. Assessment of side-effects during human studies.

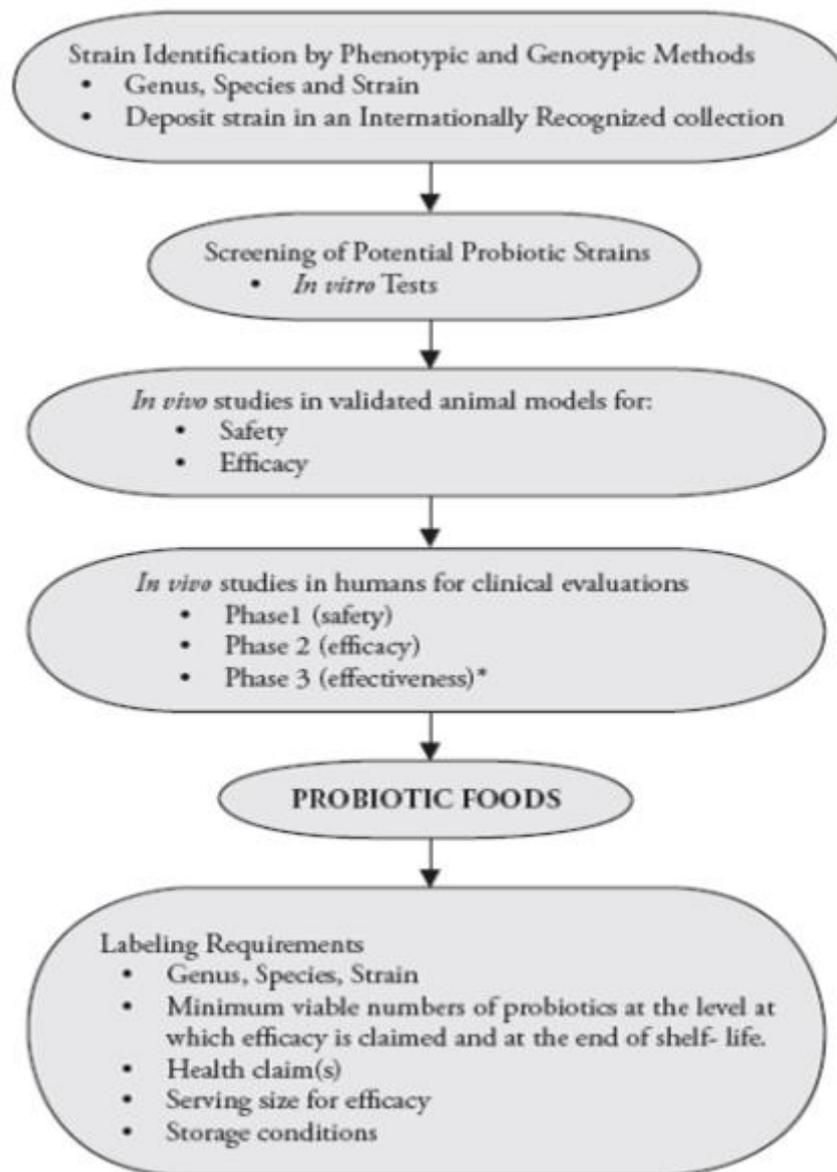
5. Epidemiological surveillance of adverse incidents in consumers (post-market survey).

6. Determination of haemolytic activity of strain is necessitated if the strain under evaluation belongs to a species with known haemolytic potential (FAO/WHO, 2002).

7. Efficacy of the novel strains and the safety status of the traditional product in which they will be incorporated must be evaluated prior to their incorporation (Donohue, 2006).

## 4. REGULATORY FRAMEWORK AND LEGISLATION FOR PROBIOTICS

A regulatory framework must be created to better address probiotic issues across the globe including safety, efficacy, fraud, labeling and claims. Probiotic products should be made more widely available, especially for relief work and to populations at high risk of morbidity and mortality. The regulatory framework for probiotics differs from country to country and also even within a country (Reid, 2001; EFFCA, 2008).



**Figure 2 Guidelines for evaluation of candidate probiotic strains** (Source: Ganguly et al., 2011).

\*Only required if a specific health claim is made

Japan was the first nation to implement regulatory system for functional foods and nutraceuticals aid with probiotics in 1991; where probiotic products are in distinct category called Foods for Specific Health Uses (FOSHU) while to make claims about efficacy, one must obtain special permission from the Ministry of Health and Welfare (MHLW) of Japan (Amagase, 2008). Secondly, in 1995 Europe established a regulatory commission known as functional food science in Europe (FUFOSE). Similarly, other nation have their

own regulations of probiotics in food and pharmaceutical products such as United States have Dietary Supplement Health and Education Act (DSHEA) and Food and Drug Administration (FDA); in Brazil, National Health Surveillance Agency Brazil (ANVISA); in New Zealand and Australia, Food Standards Australia and New Zealand (FSANZ); while in China State Food and Drug Administration (SFDA). There has been an increased influx of probiotic products in the Indian market during the last decade, as a result an initiative was

taken by the Indian Council of Medical Research (ICMR) and Department of Biotechnology (DBT), Government of India (GOI), to create guidelines for the regulation of probiotic products in the country defining a set of parameters required for a strain/product to be termed as 'probiotic' (Ganguly et al., 2011). In common, these regulations mainly address the identification of the strain, *in vitro* screening for probiotic features, and *in vivo* animal and human studies to establish efficacy, requirements for labeling of the probiotic products with strain specification, viable numbers at the end of shelf-life, storage conditions, etc., so as to help the consumers to safeguard their awareness.

## 5. CONCLUSION AND FUTURE PROSPECTS

In India, dairy based products containing live bacteria are the main vehicles of probiotics to human in addition to consumption of diverse traditional fermented foods. Non-dairy beverages would be the next food category where the healthy bacteria will make their mark. Careful selection of starters would help to enhance the quality and safety aspects of fermented food products while incorporation of bioactive compound producing strains may serve to develop value-added food products. Designer probiotics for specific treatment is a budding field of research which would reinforce the drive of using probiotics for the treatment of different ailments and human health improvement. Biotechnological tools can be helpful to enhance the technological performances of starter cultures as well as probiotic strains. The effective doses of probiotics vary from strain to strain, the food matrixes, and the host like the age, gender, healthy or immunocompromised. Thus, there is a need to better address such issues and validate the specific health claims of probiotic strains. The guidelines and regulations warrant harmonization at the global level that would ensure better quality and safety of probiotic foods for effective utilization.

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